Pharmacogenomics: What pharmacists need to know in a changing therapeutic environment.

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Where we are/ Where we want to be

- Moving from “one drug fits all” to personalized pharmacotherapy!

- Why bother?

- Estimated 100,000 deaths per year in the US due to pharmacotherapy.
- Healthcare costs of > $75 Billion / yr

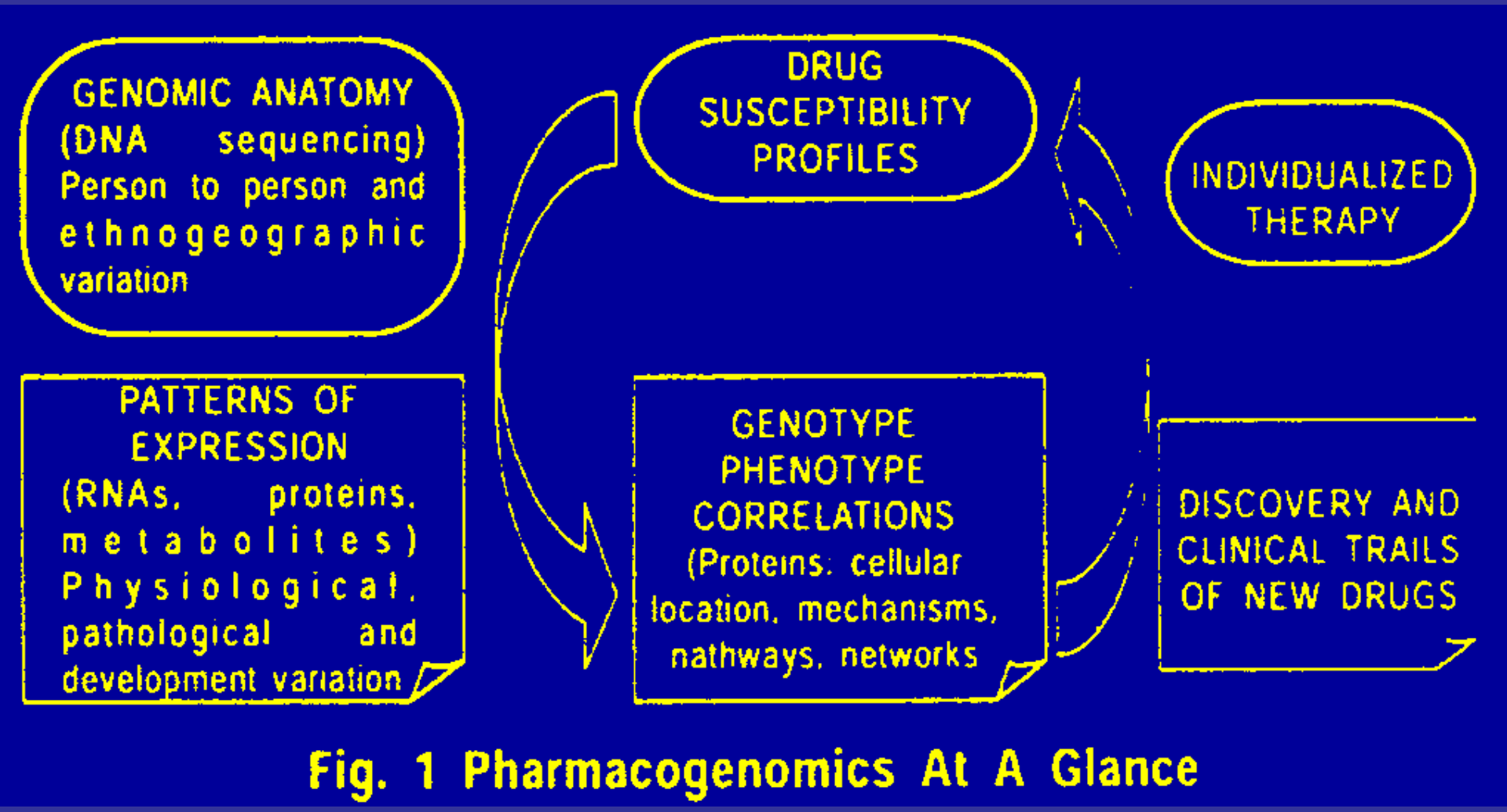


Fig. 1 Pharmacogenomics At A Glance

Genetics or Genomics?

- **Pharmacogenetics**
  - Study of how genetic differences in a SINGLE gene influence variability in drug response (i.e., efficacy and toxicity)

- **Pharmacogenomics**
  - Study of how genetic (genome) differences in MULTIPLE genes influence variability in drug response (i.e., efficacy and toxicity)

The Biology

- Cell (10-30 microns wide)
- Nucleus
- Chromosomes
- Genes
- DNA (20 angstroms in diameter)

DNA Bases

- Adenine = Thymine
- Guanine ≡ Cytosine

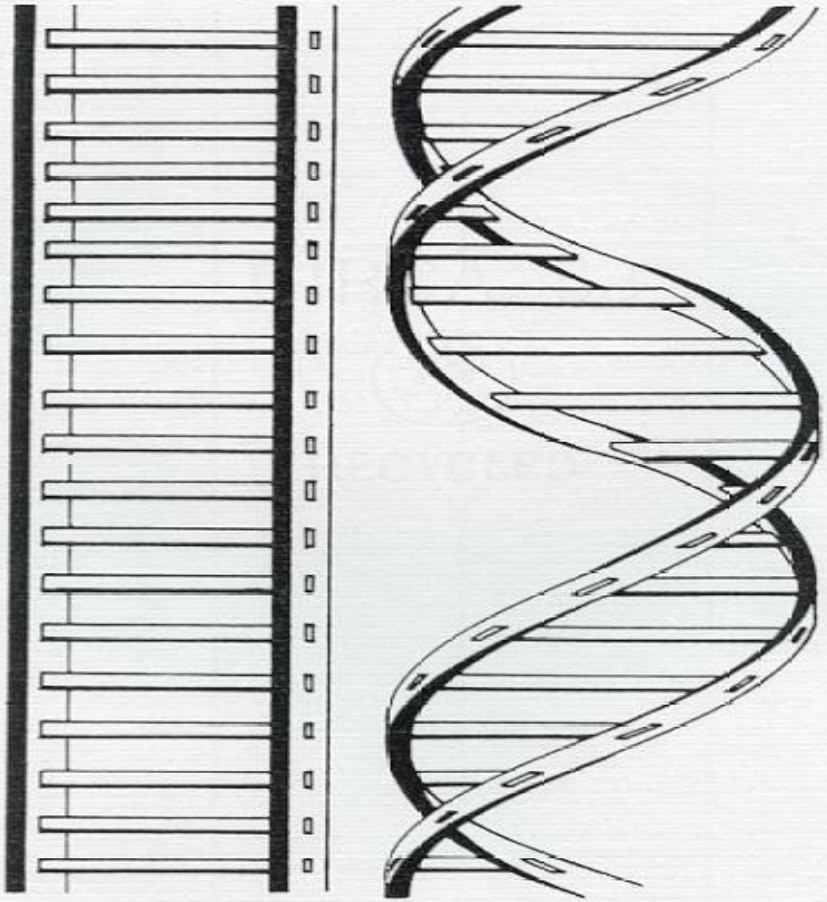
- Codon – 3 bases that code for amino acids in proteins
- 3.2 billion bases
- 35,000-45,000 discrete genes

DNA is Information

- DNA
- A, T, G, C
- Codon
- Gene
- Chromosome
- Genome

- ENGLISH
- Abcdefg….xyz
- Word
- Sentence
- Chapter
- Book

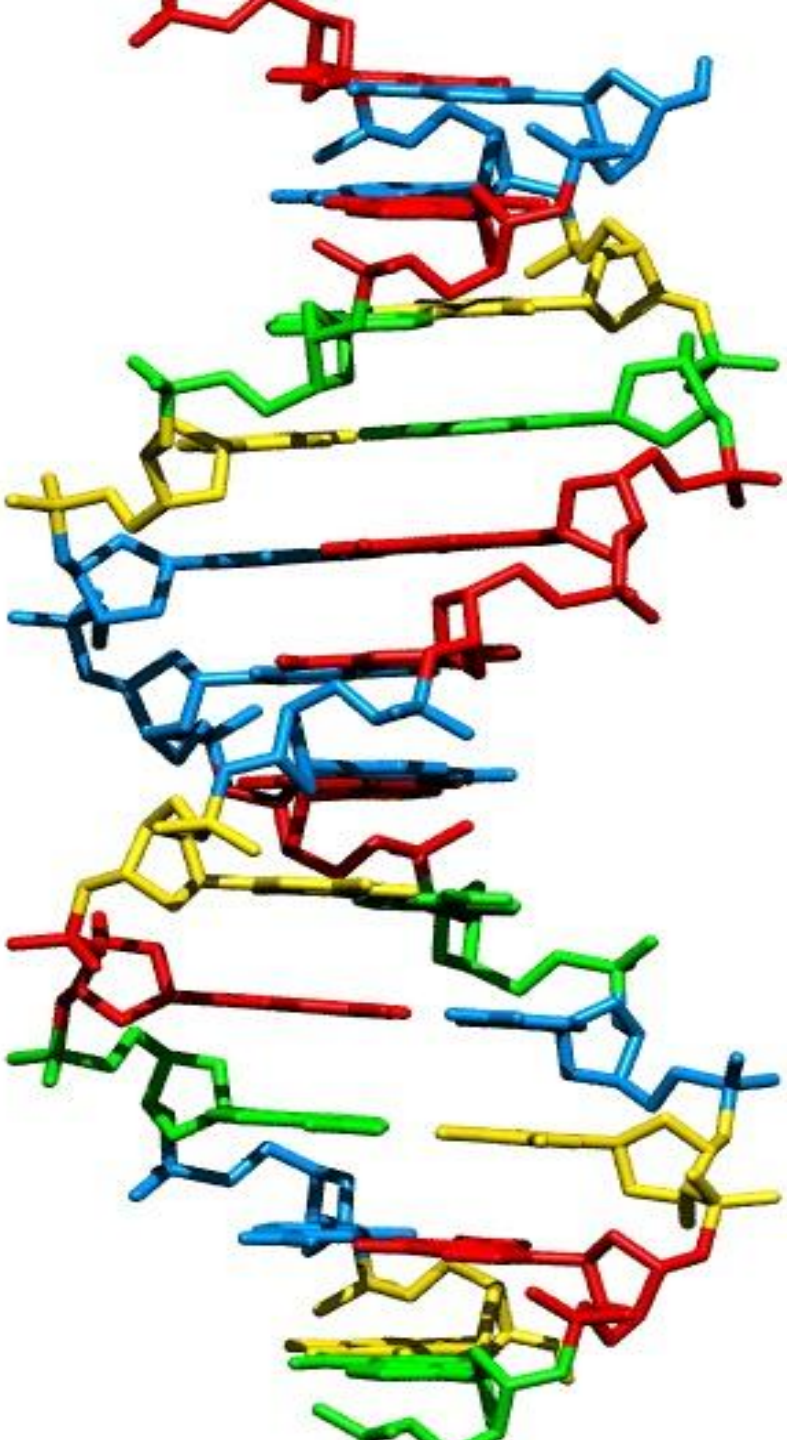
DNA



In fact, the two DNA strands are twisted around each other to make a double helix.

Francis Crick

James D. Watson



Question

- If you took all the DNA in the human body and stretched it out lengthwise how long would it be?
  - 1 mile
  - 10 miles
  - More?

130,000 X the distance to the moon and back = $3X10^{43}$ miles!!

Human Genome Project

- Began in 1990
- Funded by US Dept of Energy (DOE), US National Institute of Health (NIH) in collaboration with Britains Wellcome trust
- Originally expected completion in 2005, now 2003

Aims

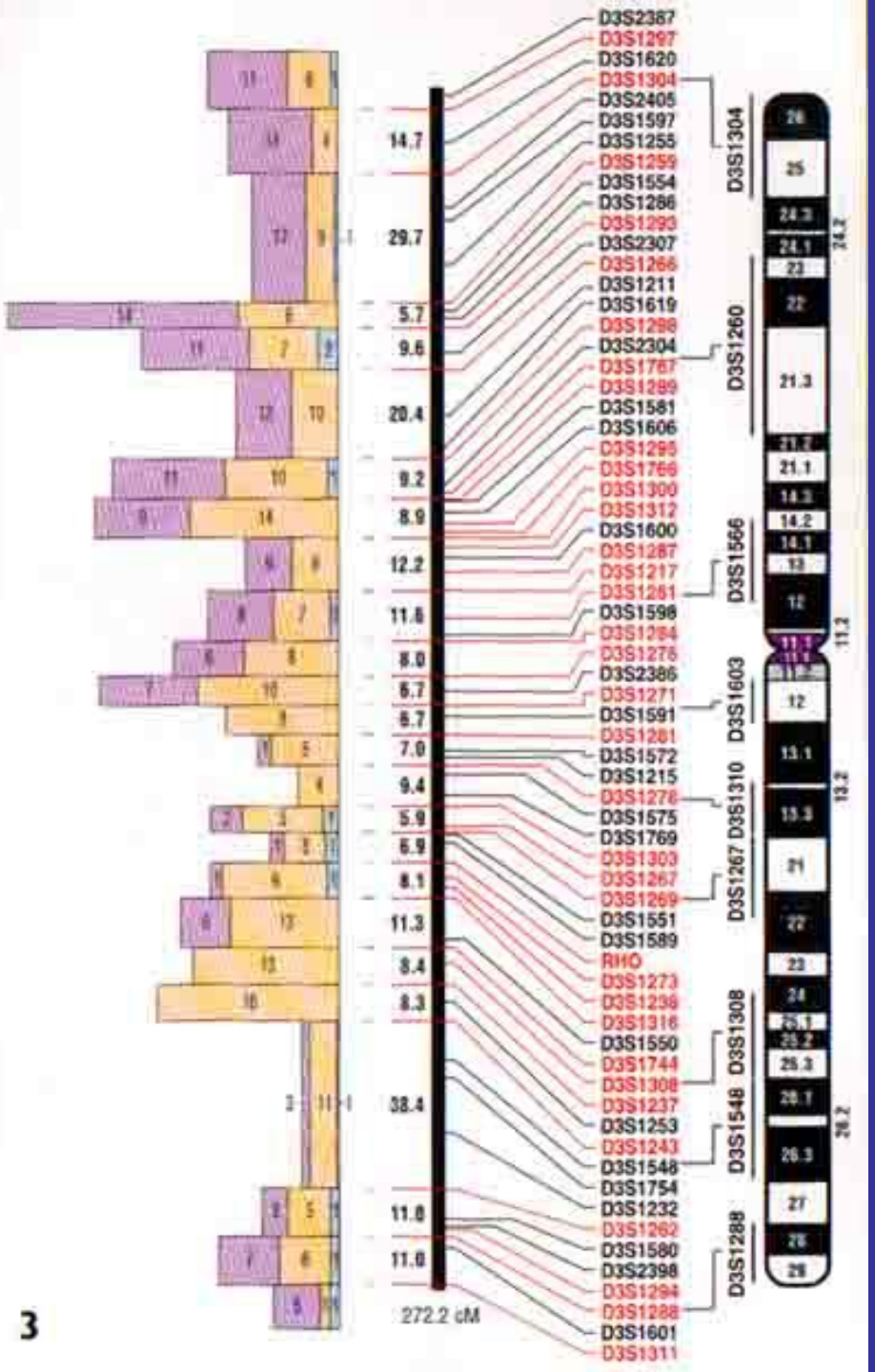
- Sequence the entire 3 billion letter human genome with high precision
- Aiming to dissect the biochemical code of each of the 100,000 or so genes that determine the physical characteristics of the human body
- Cost = $US 3 Billion

Chromosome 22- Finished

- Published in Nature, Dec 2nd 1999
- Found 545 genes and there may be as many as 1,000
- 11gaps for technical reasons (<150,000bp)
- At least 27 different human disorders associated with Chr22, eight with no known gene

Composition of the Human Genome

• Mutation/Polymorphism	1 bp
• Unit of genetic code	3 bp
• Coding sequence (exons)	3,000 bp
• Gene (exons and introns)	50,000 bp
• Chromosome	150,000,000 bp
• Human genome	3,000,000,000 bp


D3S2387
D3S1297
D3S1620
D3S1304
D3S2405
D3S1597
D3S1255
D3S1259
D3S1554
D3S1286
D3S1293
D3S2307
D3S1266
D3S1211
D3S1619
D3S1288
D3S2304
D3S1767
D3S1289
D3S1581
D3S1606
D3S1295
D3S1766
D3S1300
D3S1312
D3S1600
D3S1287
D3S1217
D3S1261
D3S1598
D3S1284
D3S1276
D3S2386
D3S1271
D3S1591
D3S1281
D3S1572
D3S1215
D3S1276
D3S1575
D3S1769
D3S1303
D3S1267
D3S1269
D3S1551
D3S1589
RHO
D3S1273
D3S1238
D3S1316
D3S1550
D3S1744
D3S1308
D3S1237
D3S1253
D3S1243
D3S1548
D3S1754
D3S1232
D3S1262
D3S1580
D3S2398
D3S1294
D3S1288
D3S1601
D3S1311
14.7
29.7
5.7
9.6
20.4
9.2
8.9
12.2
11.6
8.0
6.7
6.7
7.0
9.4
5.9
6.9
8.1
11.3
8.4
8.3
38.4
11.8
11.0
272.2 cM
D3S1304
D3S1260
D3S1566
D3S1603
D3S1310
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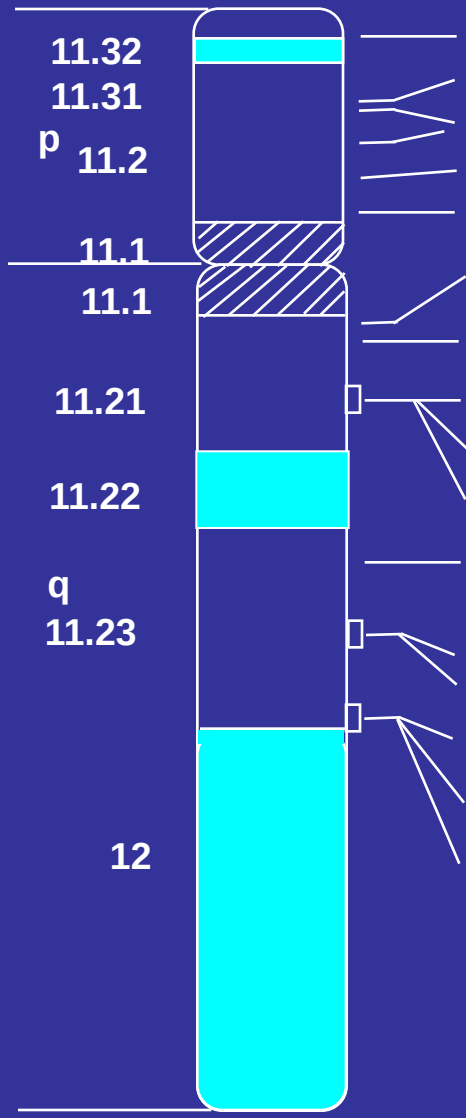
11.2
13.2
26.2
3

Major Functional (?) Genes on the Human Male Chromosome



Testis Determining Factor *(TDF)*

Gadgetry *(MAC-locus)*

Channel Flipping *(FLP)*

Catching & Throwing *(BLZ-1)*

Self-confidence *(BLZ-2)* *(note-unlinked to ability)*

Ability to Remember & Tell Jokes *(GOT-1)*

Sports Page *(BUD-E)*

Addiction to death & destruction movies *(T-2)*

Air Guitar *(RIF)*

Ability to identify aircraft *(DC10)*

Preadolescent fascination with Arachnida & Reptilia *(MOM-4U)*

Spitting *(P2E)*

Sitting on the john reading *(SIT)*

Inability to express affection over the phone *(ME-2)*

Selective hearing loss *(HUH?)*

From *Science* 261: p 679, 1993
Jane Gitschier

The Foundation of Pharmacogenomics: Differences in the Genetic Code Between People

- **Mutation**: difference in the DNA code that occurs in less than 1% of population
  - Often associated with rare diseases
    - Cystic fibrosis, sickle cell anemia, Huntington's disease

- **Polymorphism**: difference in the DNA code that occurs in more than 1% of the population
  - A single polymorphism is less likely to be the main cause of the disease
  - Polymorphisms often have no visible clinical impact

Single Nucleotide Polymorphisms (SNP)

- Pronounced “snip”
- Single base pair difference in the DNA sequence

- Over 2 million SNPs in the human genome

SNP's

- Single nucleotide polymorphisms
- Consequences:
  - 1. Silent – no AA change
  - 2. Variant protein formed- altered function?
  - 3. Exon/Intron SNP's – truncated protein – bad?
  - 4. Regulatory regions – alter gene expression
    - (duplications/amplifications)

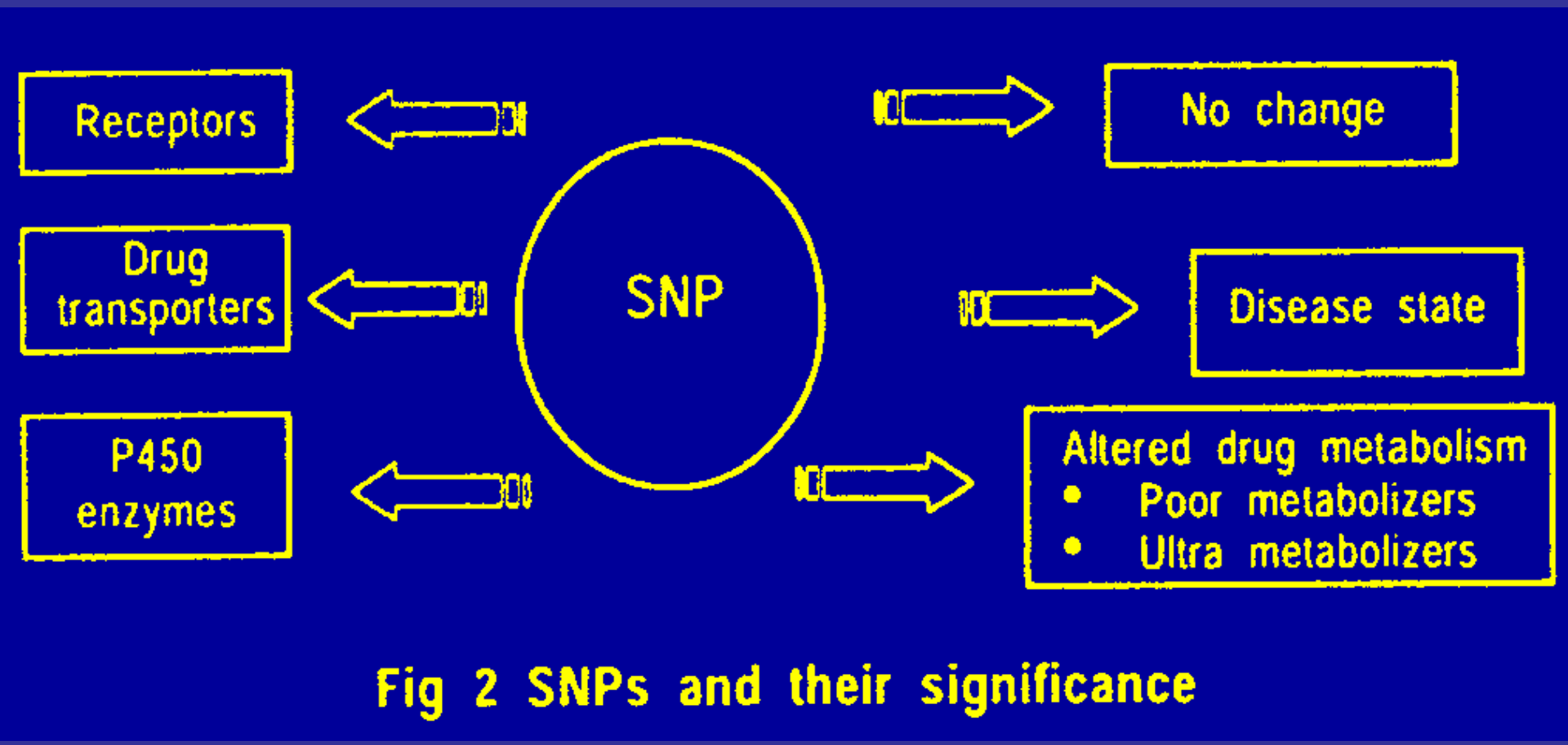


Fig 2 SNPs and their significance

Genetics Terminology

- **Alleles** = different DNA sequences at a locus
  - Codon 389 β_1-AR
    - Arg (0.75)
    - Gly (0.25)

- **Genotype** = pair of alleles a person has at a region of the chromosome
  - Codon 389 β_1-AR
    - Arg389Arg
    - Arg389Gly
    - Gly389Gly

Gene Arrays

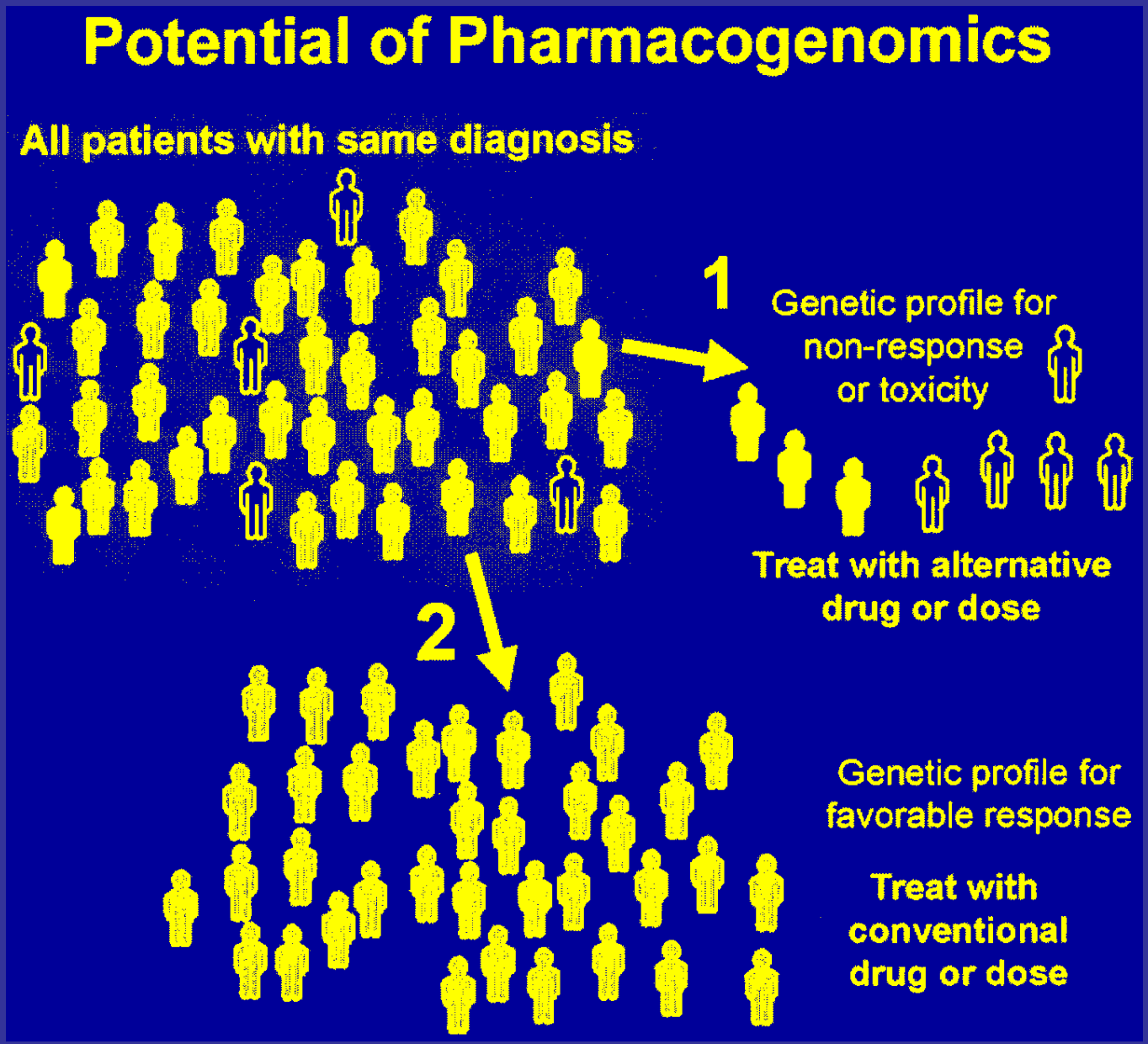
- 64,000 gene clones per 1 sq inch

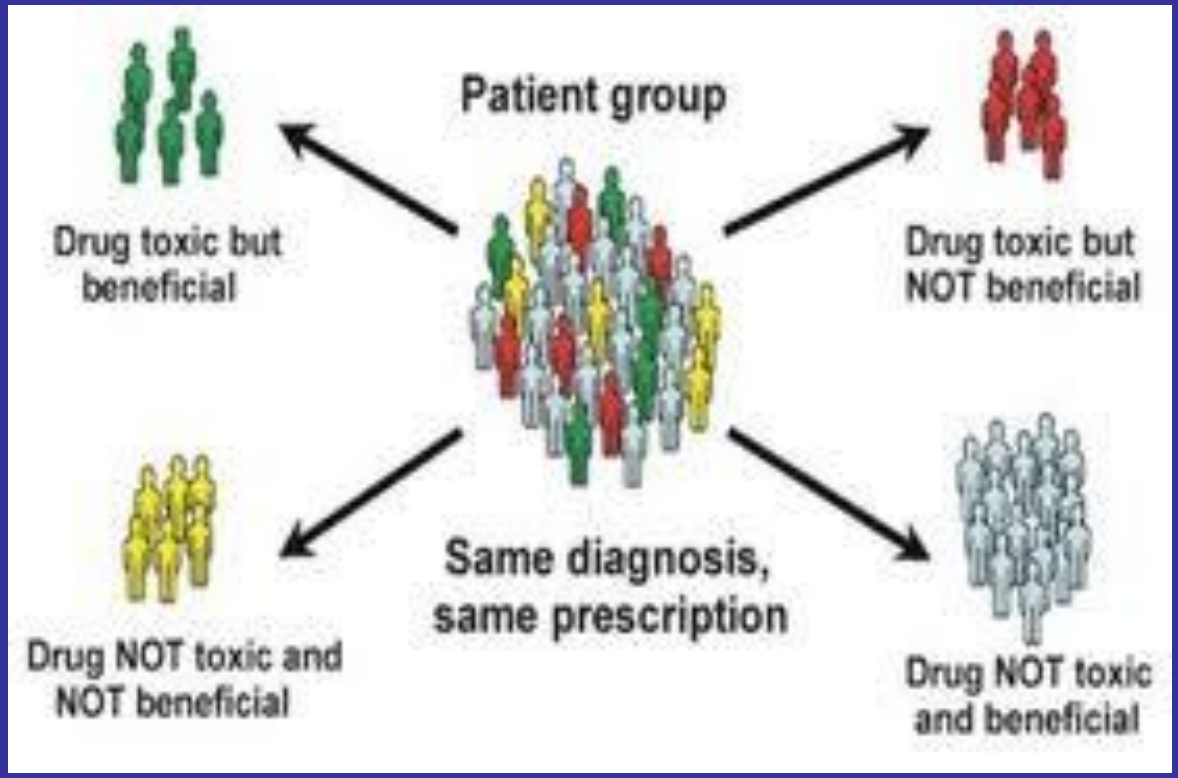
- 48 hrs today!
- 20 yrs with standard Western Blot analysis

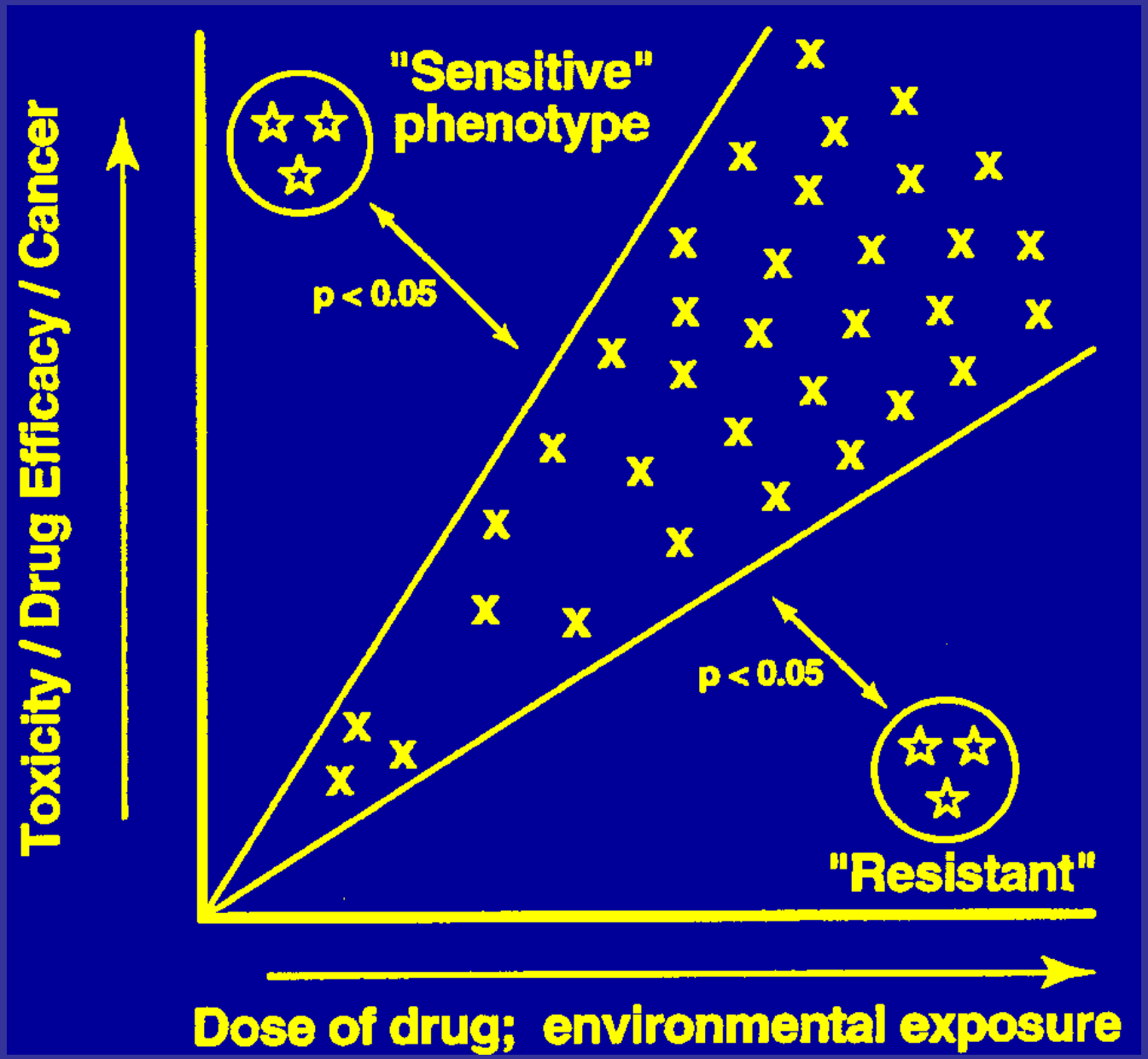
Adverse drug effects

- IND Phases 1, 2, 3
- NDA submission
- Phase 4 – postmarketing surveillence
- “rare” adverse effects seen in Phase 4

Potential of Pharmacogenomics

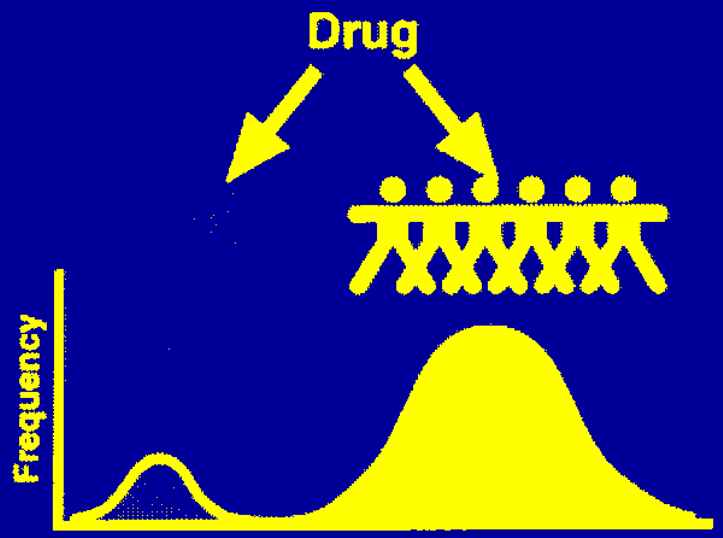



Patient group
Drug toxic but beneficial
Drug toxic but NOT beneficial
Same diagnosis, same prescription
Drug NOT toxic and NOT beneficial
Drug NOT toxic and beneficial


"Sensitive" phenotype
p < 0.05
p < 0.05
"Resistant"
Toxicity / Drug Efficacy / Cancer
Dose of drug; environmental exposure

Pharmacogenetic Discovery

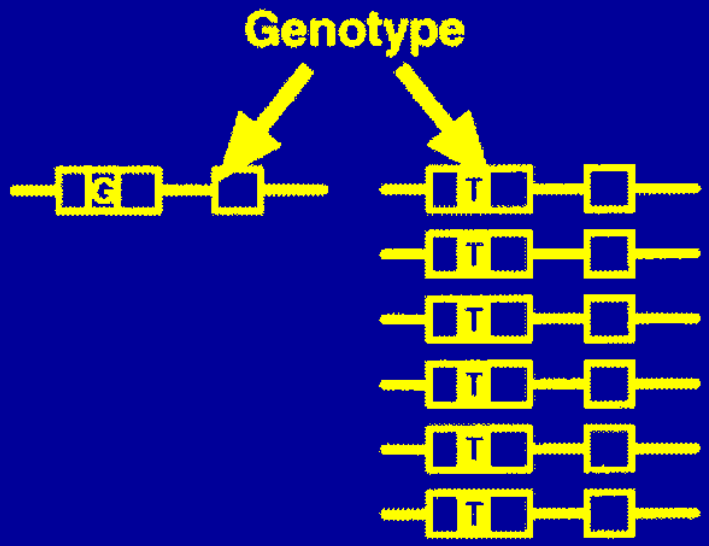
Pre-Genomics



Genotype

Compare genetic polymorphisms in *phenotypic* groups

Post-Genomics



Compare phenotypes in *genotypic* groups

ADME

- Absorption
  - Carrier-mediated transport

Drug metabolism by the major families of CYP450 enzymes

CYP450 isoform	% of drugs metabolized
CYP3A4	55
CYP2D6	20
CYP2C19	15
CYP1A2	5
CYP2E1	1
Others	4

CYP2D6 Polymorphisms

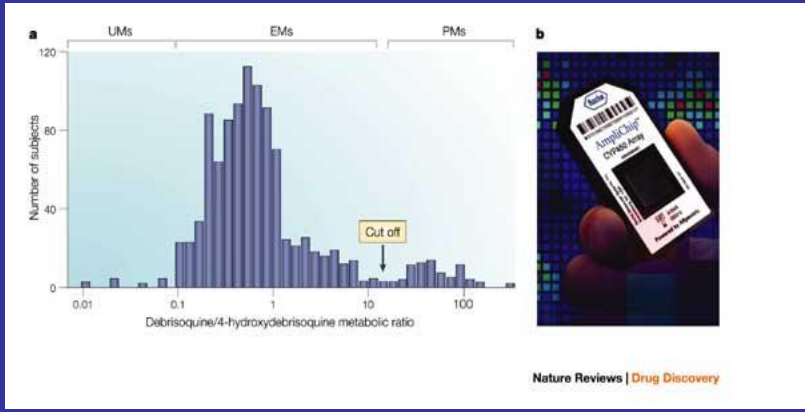
- CYP2D6 is responsible for the metabolism of a number of different drugs
  - Antidepressants, antipsychotics, analgesics, cardiovascular drugs

- Over 100 polymorphisms in CYP2D6 have been identified

- Based on these polymorphisms, patients are phenotypically classified as:
  - Ultrarapid metabolizers (UMs)
  - Extensive metabolizers (EMs)
  - Poor metabolizers (PMs)

U.S. Food and Drug Administration

Roche AmpliChip Cytochrome P450 Genotyping test and Affymetrix GeneChip Microarray Instrumentation System - K042259



ADME

- Metabolism
- e.g., Codeine
- O-demethylation to morphine
- CYP2D6
- Caucasians - 2-10% - ineffective
- Chinese pts also tend to produce less M & are less sensitive to M (maybe decr. M6G production – phase II metab.)

CYP2D6 Polymorphisms and Psychiatric Drug Response

- Increased rate of adverse effects in poor metabolizers due to increased plasma concentrations of drug:
  - Fluoxetine - death in child attributed to CYP2D6 poor metabolizer genotype
  - Side effects of antipsychotic drugs occur more frequently in CYP2D6 poor metabolizers
  - CYP2D6 poor metabolizers with severe mental illness had more adverse drug reactions, increased cost of care, and longer hospital stays

Atomoxetine

- Treatment of attention deficit hyperactivity disorder (ADHD)
  - CYP2D6 poor metabolizers have 10-fold higher plasma concentrations to a given dose of atomoxetine compared with extensive metabolizers
  - Approximately 7% of Caucasians are poor metabolizers
  - Higher blood levels in poor metabolizers may lead to a higher rate of some adverse effects of atomoxetine

CYP2C19 and Proton Pump Inhibitors

- Proton pump inhibitors are used to treat acid reflux and GI hyperacidity
- Ulcer cure rates using omeprazole and amoxicillin by CYP2C19 phenotype:

	Cure Rate
- Rapid metabolizers	28.6%
- Intermediate metabolizers	60%
- Poor metabolizers	100%

Furuta, T. et. al. Ann Intern Med 1998;129:1027-1030

Warfarin and CYP2C9

- Widely prescribed anticoagulant drug used to prevent blood clots
- Narrow range between efficacy and toxicity
- Large variability in the dose required to achieve therapeutic anticoagulation
  - Doses vary 10-fold between people
- CYP2C9 is the enzyme responsible for the metabolism of warfarin
- SNPs exist in CYP2C9 gene that decreases the activity of the CYP2C9 metabolizing enzyme

TPMT

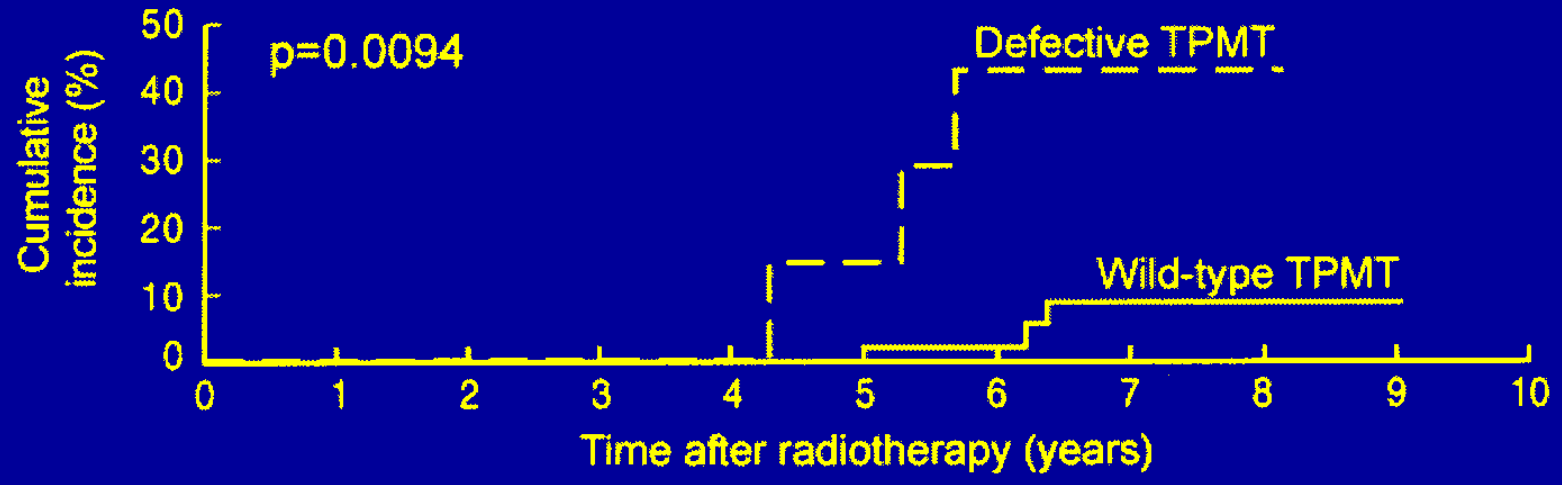
- Thiopurines are prodrugs
  - Azathiopurine
  - Thioguanine
  - Mercaptopurine
- Activated to thioguanine nucleotides
- TX =
  - Dermatological
  - Transplantation
  - IBD
  - Rheumatoid arthritis
  - Acute lymphoblastic leukemia

TPMT

- Metabolized by thiopurine-S-methyl
- transferase (TPMT) via S-methylation.
- Produces non-toxic metabolites
- If there is a lack of TPMT – toxicity results – severe & life threatening
- TPMT is highly polymorphic
- 89% hi, 10% intermed., 0.3% low activity

Interactions of Genetic Polymorphisms and Treatment May Result in Adverse Effects

B.



Estimated cumulative incidence of radiation-associated secondary malignant brain tumor in patients treated with concomitant mercaptopurine and irradiation.

TPMT

- TPMT*2 = G238C (codon18 ALA/PRO)
- TPMT*3A = G460A (codon 154 ALA/THR)
  A719G (codon 240 TYR/CYS)
- TPMT*3C = A719G

- The 3 alleles are associated with accelerated proteolysis of TPMT

COMT

- Catechol-O-methyltransferase
- Metabolizes levodopa and alpha-methyldopa
- African and East Asian populations have higher activities

Pharmacodynamics

- Receptor polymorphisms
  - synthesis rate
  - activity level
  - desensitization

Variability in the Response to Albuterol

- Interpatient variability:
  - Gender
  - Race
  - Concomitant diseases
  - Age
  - Interactions with other Rx
- Intrapatient variability:
  - Desensitization
  - Tachyphylaxis
  - Tolerance

Beta-2 Adrenoceptors

- Dynamic !
- Up-regulation & down-regulation
- Tachyphylaxis
- Agonist-induced uncoupling of receptors from the G-s binding protein

Beta-2 Adrenoceptor Allele Distribution

- ARG 16
  - Homo ARG16/ARG16 15%
  - Hetero ARG16/GLY16 38%
  - Homo GLY16/GLY16 45%
- GLN 27
  - Homo GLN27/GLN27 26%
  - Hetero GLN27/GLU27 49%
  - Homo GLU27/GLU27 22%

Polymorphism and Albuterol

- Asthmatic pts.
- Beta-2 adrenoceptor genotyping
  - 5ml blood
  - Extract genomic DNA
  - PCR
    - Allele-specific nucleotide probes
    - hybridization

Albuterol Study Design

- Pts with bronchial asthma
- Beta-2 adrenoceptor genotyping
- 8 mg dose p.o.
- Determined:
  - % FEV1 change
  - Albuterol plasma concentrations

Heart Failure

- Beta-2 adrenoceptor polymorphism

- Codon 164 (THR>ILE) – 42% 1yr survival
- Codon 164 (THR>THR) - 76% 1yr survival

- Aggressive treatment strategies?

Alzheimer's Disease

- Tacrine therapy

- APOE-4/4 poor response (40%)
- APOE-4/3 good responders (83%)

Land a man on the Moon!

- Space travel
- Then and now

Drug Development

- Smarter drug development !
- More economical !
- Faster !
- Less adverse reactions !

Pharmacogenomics and Public Health

- TPMT SNP allele frequency distribution
- Toxicity low in Japanese & Chinese
- Toxicity high in Ghana and Kenya
  - Ghana
    - Ewe & Fanti (lowest levels of activity)

CONCERNS

- Health Insurance coverage ?
  - Standard of care
- Privacy Issues ?
  - Insurability ?
  - Discrimination ?

Advances in Science and Technology

- 1st Law- When a distinguished but elderly scientist states that something is possible, he is almost certainly right. When he states that something is impossible he is very probably wrong.

- Sir Arthur C. Clarke

Advances in Science and Technology

- 2nd Law- the only way of testing the limits of the possible is to venture beyond them into the impossible.

- Sir Arthur C. Clarke

Advances in Science and Technology

- 3rd Law- any sufficiently advanced technology is (initially) indistinguishable from magic.

- Sir Arthur C. Clarke